DK 637.0
Milchwirtschaftliches
Ordnungssystem
Bibliotheca Lactis 21

DAVID R. HOMER and ARTTURI I. VIRTANEN

Cow's milk cholesterol — Studies on the milk of cows on normal and protein-free feeds

Laboratory of the Foundation for Chemical Research,
Biochemical Research Institute, Helsinki, Finland

Cholesterol is a quantitatively minor and, apart from sterol analyses used in the examination of mixtures of fats and oils, infrequently investigated component of milk fat. Table I is a summary of the figures reported to date for the concentration of

Table 1. The concentration of cholesterol in cow's milk and milk fat

Reference	Milk fat %	Total cholesterol mg/100 ml milk [1]	Total cholesterol mg/100 g fat	Cholesterol ester, % of total
30				0
4				0
31	4.1	13.8	335*	
32	3.7	12.6	345*	100
5				35
33	3.9	12,7	330*	
34		11.5		
6		11.8	300	0
1	3.6	22.4	640	
35	3.6	12.9	364*	0
36		11		
37	3.1	10.4	330	
38		15.1	390	
11	3.7	11.4	315*	0
39				9
12		13.5**		
40	3.7	13.4	360*	
41		11.5		
7		17.0		
42	3.8	14.8	395*	
9	4.0	15.0	375	15
2		28		0
13	3.2	11.6	365*	0.2
15		11.2		
18,54				10
43	3.6	12.5	350*	0.5
44				6

[1]) units given variously as "mg %", mg/100 ml or mg/100 g milk.
* 100 X (cholesterol, mg/100 ml milk) ÷ milk fat %.
** for foreign breeds in India. For Sindhi cows, 10—50 ppm. cholesterol in milk.
The table includes data on the proportions of free and esterified cholesterol in butter. In some instances, approximate average figures only are quoted.

cholesterol in milk and milk fat. The mean values of these figures, excluding the results of DAM[1] and KRITCHEVSKY and TEPPER[2], are 12.8 mg cholesterol per 100 ml milk and 345 mg per 100 g milk fat.

Cholesterol is determined usually by precipitation with digitonin, followed by gravimetric or colorimetric estimation of the cholesterol digitonide. Precipitation before and after saponification allows the estimation of the proportions of free and esterified cholesterol. Findings pertinent to the determination of cholesterol in milk fat have been reviewed [51, 52]. On the microscale with colorimetric methods, the direct estimation of esterified cholesterol of milk fat is preferred to measurement as the difference between total and free cholesterol.

The full sterol composition of milk fat has yet to be elucidated, and though cholesterol is known to be the main component other sterols in small quantities may be recovered by the analytical procedures used for cholesterol. Lanosterol has been identified in the unsaponifiable matter of butterfat, the yield of material isolated being some 2 % of the cholesterol isolated, and it was reported to give a precipitate with digitonin within one hour[3]. Analyses by gas chromatography of the sterol fraction of butterfat unsaponifiables[53] and by thin-layer chromatography of sterol acetate prepared from butterfat sterol digitonide[52] revealed only one sterol, cholesterol. No other analysis of butterfat sterols appears to have been made.

The fat globules of milk do not contain the whole of the milk cholesterol. There were early proposals that part of it may be associated with the milk protein [4, 5], and later studies showed that such cholesterol accounted for some 18 % of the total in milk[6]. Other work on protein-bound cholesterol has been reported [7, 8]: cholesterol associated with casein was equivalent to 1.5 mg per 100 ml whole milk[7]. According to Mulder and Zuidhof, cholesterol other than that present in the fat globules (in the fat phase or its interfacial material) accounts for some 14 % of the total milk cholesterol[9]. Thompson et al. give figures for the amounts of cholesterol and cholesterol ester in lipid isolated from milk fat globule membrane[10]. The extraction of the fat from milk by standard procedures employing solvents should recover both fat globule- and protein-bound- cholesterol. The concentration of cholesterol in the lipid of the fat globule membrane and in the protein bound lipid is relatively high and the concentration in the bulk lipid phase relatively low. Reported figures for cholesterol in butter or butterfat average about 250 mg per 100 g fat.

There is a paucity of detailed information about the effects of feeding on the concentration of cholesterol in milk, and about any differences there may be due to breed, season, stage of lactation and so on. Dam's cholesterol feed seemed to have some effect on the concentration of cholesterol in milk fat, but the data were too scanty to allow any definite conclusion[1]. In tests with three breeds, Nataf et al. found no seasonal differences in milk cholesterol values. There was a significant between-breed difference during the winter only, and a significant positive relationship between the cholesterol and fat contents of the milk[11]. Other investigators found no significant seasonal or inter-breed variations in the level of cholesterol in herd milk [12, 13, 14]: Steger reported a mean value of 12.4 mg cholesterol % for Jersey cows (four cows), the means for two other breeds being 10.8 and 10.3[15]. Highest cholesterol values were measured during early or at mid lactation, with a rise later towards the end of the lactation[12, 15]. A study of the cholesterol content of milk obtained at the beginning and at the end of milking has been made[16].

In 1964 Hartmann and Lascelles described a study of the uptake of blood plasma lipid by the cow mammary gland. They found that neither cholesterol nor cholesterol ester was taken up by the lactating or non-lactating gland[17]. Thus cholesterol sufficient to account for a daily output of a gram or more is synthesised within the udder. This synthesis would appear to be for the purpose of milk fat synthesis as a whole, since Patton and McCarthy have found evidence that cholesterol esters play an active role in milk fat synthesis, possibly in the transfer of fatty acids from a common "pool" to form the triglycerides of milk fat[18, 19, 20, 22]. Part of this mammary gland machinery, containing thus cholesterol, for synthesis of fat is secreted as part of the milk[23, 24].

There is considerable evidence that the long-chain fatty acids which, bound as glycerides, are the main components of milk fat are derived in part directly from the blood stream and in part from synthesis, from acetate and β-hydroxybutyrate, within the mammary gland.[50] Riis and Moustgaard have presented data according to which the contribution from the two sources may be about the same[27]. These findings are summarised in Figure I.

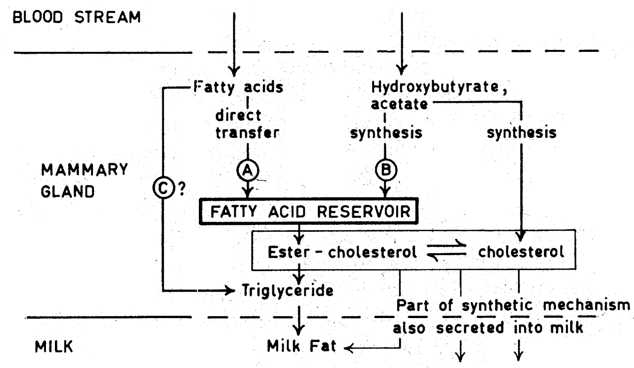

Figure I. A conception of the part that cholesterol may play in milk fat synthesis, as suggested by the recent work of Patton, McCarthy and Hartmann and Lascelles. For a discussion of the proposed route C see text.

In addition, Riis' findings suggested that in one breed of dairy cow (Jersey) the amount of fat synthesised from acetate in the udder is relatively large while that formed from the plasma lipids was small, whereas with other breeds (Red Danish and Black Pied Danish) more milk fat was derived from plasma lipids[28]. If this were so, we would not expect, however, any differences in the proportions of cholesterol secreted in the milk by these different breeds, as both routes A and B (Figure I) terminate in the common fatty acid "pool". But if we propose an alternative route (C, Figure I), partly or completely replacing route A, by which fatty acids are incorporated into the precursors of milk fat without the intervention of cholesterol, we might expect an alteration in the balance between route A/C and route B to cause a change in the concentration of cholesterol in the milk fat secreted by the gland. At the same time, we would not expect a significant change in the proportion of esterified cholesterol in the total cholesterol of the milk.

The data summarised above show that inter-breed milk cholesterol differences are slight or absent, and definitely no consistent long term differences associated with different feeds have previously been established.

Measurements of milk cholesterol have been made in this laboratory over a period of several years. Milk samples were taken from cows on normal feed and cows receiving an experimental feed. This feeding experiment with a number of Ayrshire cows was begun in October 1961. The cows received a protein-free feed composed basically of purified carbohydrate, urea, ammonium salts, minerals, fat-soluble vitamins and a small amount of vegetable oil. A good production of milk of high fat and protein content has been maintained during the five year period of the experiment to date, covering, with some of the test cows, several successive lactations[29].

Experimental. Test milk samples were brought to the laboratory every fortnight for full analysis, and herd samples (Ayrshire cows) were collected from nine farms in the Helsinki or Lahti areas. Included also was a test using winter feed modified to reduce the fat intake considerably (farm 9). Further details are given in Table II. The ages of the test cows at the commencement of the cholesterol measurements (April 1963) were: — No. 1 5 years, No. 3 6 yrs., No. 4 5 yrs., No. 5 2 yrs. and No. 6 8 yrs.

Cow 5 was a heifer at the time of adaptation to the test feed; the others had calved at least twice before adaptation. Milk from the 22 cow herd on farm 1 was sampled further over a period of 6 months for a check on the variation of milk cholesterol values with stage of lactation.

Milk fat %. This was determined by the Röse-Gottlieb or Gerber methods: the results are given as weight percentages.

Milk fat samples for cholesterol determination: Röse-Gottlieb fat. A few butterfat samples also, prepared by centrifuging milk or cream, were analysed.

Determination of total cholesterol

Apparatus Glass centrifuge tubes, eg. 16 x 85 mm, 200 mm stirring rods.

Reagents Cholesterol, pure. A sample of "Bacto-Cholesterol" (Difco Laboratories Inc., Detroit, Michigan, USA) had a melting point of 149—150 °C and thin-layer chromatography tests using the solvent systems of Williams et al. [45] showed the absence of material of R_f differing from that of cholesterol.

Standard cholesterol solution (prepare fresh frequently), ferric chloride solution, colour reagent and ethanol-acetone mixture as detailed by Brown et al.[46]. Working standard cholesterol solution: 0.030 mg/ml in glacial acetic acid.

10 % v/v acetic acid solution.

Stock 1 % ethanolic digitonin reagent (Digitonin, Krist., Merck, Darmstadt, Germany, was found satisfactory). Working 0.5 % digitonin reagent: dilute 1 % reagent with water; prepare fresh every few weeks.

3.5 % ethanolic KOH: dissolve about 0.8 g p. a. KOH in 20 ml ethanol.

Method Tare the centrifuge tubes and calibrate them at 2 ml. Weigh the milk fat sample (about 20 mg) into the bottom of the centrifuge tube, add 1 ml 3.5 % ethanolic KOH and heat for one hour in a water bath held at 75—80 °C. To the hot residue add 1 ml water and ethanol to the 2 ml mark, add dropwise about 0.25 ml 10% acetic acid followed by 2 ml ethanol-acetone and mix with a stirring rod. Add

0.3 ml 0.5 % digitonin reagent (for 0.1 mg cholesterol this gives a molecular ratio digitonin: cholesterol of 15 : 1), mix thoroughly, leaving the rod in the tube, allow to stand overnight and centrifuge at 1000 xg 10 min. Decant the supernatant and drain the tube by inverting and standing it on filter paper. Remove residual supernatant with a rolled filter paper. Pipette in 3 ml glacial acetic acid and prepare blank and standards, the latter from 3 ml working standard cholesterol solution. Assemble all the tubes of the batch and stand them in warm water until the digitonide precipitate has dissolved. Cool the tubes to room temperature before colour development and reading as described by Brown et al.[46]. Maximum colour development is attained in a few minutes and the colour complex is stable at room temperature for several hours at least. Verify that the colorimetric response is linear and calculate the sample cholesterol from the spectrophotometer reading of the sample and standards in the same batch.

Reproducibility of the results In practice the cholesterol content of any one fat sample was determined twice, in different batches, and the figure was generally repeatable to within 5 % of the mean. The use of the stirring rods, as recommended by Sperry and Webb[47], improved the repeatability.

Colorimetric response This was found to be linear, whenever tested, for amounts of cholesterol up to at least 0.12 mg.

Length of saponification One sample was analysed using 0.5 and 1 hour saponification times. The cholesterol figures were 335, 345, 355 and 350, 355 360 mg/100 g fat respectively.

Recovery of added cholesterol and cholesterol ester Previously analysed samples were repeated after addition of 20 μg cholesterol or 18 μg cholesterol as ester. Recoveries were 21, 20, 18, 19, 20, 20, 20, 21 and 22 μg cholesterol and 20, 20, 17, 20, 18, 17, 18, 16 and 16 μg cholesterol as ester.

Comparison with a gravimetric procedure 3 butterfat samples together with data on their cholesterol contents, as found by saponification[48] and removal of cholesterol from the unsaponifiable matter[49], were kindly supplied by Dr. D. P. Schwartz. These samples were further analysed using the colorimetric procedure described above: the results, in mg cholesterol per 100 g fat, were: —

	Gravimetric method	Colorimetric method
Herd milk	256	248
Test milk 1	331	330
Test milk 2	377	357

Lanosterol interference 70 μg portions of lanosterol (96 %, Sigma Chemical Company, St. Louis, Missouri, USA) were saponified etc. as for the determination of cholesterol. No precipitate of digitonide was obtained after overnight standing, and in repeat tests in the presence of cholesterol (added as the pure compound) lanosterol did not co-precipitate with the cholesterol digitonide. An acetic acid solution of lanosterol developed with the colour reagent gave a yellow-brown colour with an absorbance peak at 490 mμ. The absorbance at 560 mμ was only about one-sixth of that of an equal weight of cholesterol.

Determination of esterified cholesterol The method is an adaption of the standard procedure of Hirsch and Ahrens for the resolution of natural lipid mixtures by silicic acid column chromatography[21].

Apparatus Glass columns prepared from a 70 mm length of 10 mm id. tubing fused to a reservoir consisting of a 70 mm length of 27 mm id. tubing.

Reagents Ferric chloride and colour reagents as for the determination of total cholesterol.

Silica gel, 0.05—0.20 mm (eg. Kieselgel „für die Chromatographie", Merck, Darmstadt, Germany)

Petroleum ether, boiling range 40—60 ° C, distilled.

Ethyl ether, peroxide-free, distilled and dried.

1% and 4% v/v ethyl ether in petroleum ether.

Cholesterol esters, pure. BDH (Poole, England) cholesterol acetate and oleate were found satisfactory. The latter was stored under nitrogen at 0—5°C and solutions were used immediately after preparation. Weigh out 83 mg acetate or 127 mg oleate and dissolve in a few ml chloroform. Transfer to a 50 ml graduated flask, using chloroform to a final volume of about 10 ml, and dilute to the mark with glacial acetic acid. A 25-fold dilution of this solution with glacial acetic acid gives a 60 μg/ml solution of cholesterol as ester suitable for direct colour development.

Method Activate the silica gel overnight at 100°C, slurry in petroleum ether and transfer to the column, over a small cotton- or glass-wool plug, to give a gel column height of 50 mm (2 g gel). Load the column with milk fat or cholesterol ester and establish the elution pattern, using 1 % and then 4 % ether in petroleum ether, for quantitative recovery of the ester. In this laboratory 100 mg milk fat samples were analysed, the columns were eluted with 40 ml 1 % and 12 ml 4 % ether in petroleum ether and appropriate cuts were taken as blanks and checks on the elution of ester. Remove solvent from the recovered ester fraction and take up in 3.0 ml glacial acetic acid. Colorimetry is as for total cholesterol determination.

Colorimetric response This was linear up to the maximum tested (60 μg cholesterol) and some 10 % greater than that of cholesterol. It has been reported that the colorimetric response of cholesterol, using the ferric chloride-sulphuric acid method, is the same whether or not the sterol is esterified[25, 26].

Reproducibility of the results In practice a single estimate only of the cholesterol ester per sample was made. Results of check parallel runs were: —

	Cholesterol as ester, mg/100 g fat	
Sample 1	18	18
2	17	18
3	24	23
4	43	40

Recovery of added ester 19 μg cholesterol as ester was added to each of a number of samples previously analysed. The recoveries were 18, 19, 19 and 21 μg.

Results and Discussion

Total and esterified cholesterol, normal and test feeds (Table II)

Though there was considerable herd and test cow variation, the concentration of cholesterol in the whole milk or milk fat of the test cows was significantly higher than the corresponding values for the normally-fed cows, which themselves are close to those reported elsewhere (Table I). The increases in the cholesterol concentration in the fat and whole milk as a result of the test feed average 30 and 69% respectively. Since the lipid intake of the test cows was less than that of normally-fed cows, there appears to be an inverse relationship between the lipid intake and the milk cholesterol content (see, however, Table III). The low-fat feed (farm 9) also gave high cholesterol values. On farm 1, the milk fat cholesterol value with silage feed is significantly lower (P less than 1 %) than that with pasturage.

Despite these differences the level of esterified cholesterol as a proportion of the total cholesterol remained almost constant, being 5 % for normal feed and 6 % for the test feed. Thus it appears that for such comparative purposes the level of total cholesterol alone provides sufficient indication of the cholesterol status of the sample.

These results are consistent with the supposition that the proportion of test milk fat derived from ready-synthesised blood lipid is relatively small — other supporting data are the low concentration of lipid in the blood of the test cows together with a volatile fatty acid level which is approximately normal, and the low concentration of C_{18}-fatty acids in the milk fat of the test cows[29] — provided that there is a mechanism, in addition to that involving cholesterol, whereby mammary gland fatty acid becomes milk lipid without the intervention of the cholesterol $\rightleftarrows$ cholesterol ester system (Figure I).

Test feed : effect of oil ration on milk cholesterol Table III shows how the milk cholesterol values of the five test cows remained largely unchanged during the period when the milk samples were taken and

Table II. Cholesterol content of the milk of cows on normal and protein-free feed

Normal Feeding Farm No.	No. of milking cows	Feed	No. of samples	Period covered	Fat % in milk	Milk Cholesterol Total mg/100 g fat	Milk Cholesterol Total mg/kg milk	Ester mg/100 g fat
1	26—29	AIV silage	23	Feb-May'63, Oct'63-May'64, Apr-May'65	4.5	300 (5)	135 (3)	16
1	26—29	Pasture	16	May-Sep'63, May-Sep 64, May-June'65	4.2	330 (8)	140 (4)	23
2	3	AIV silage	19	Jan-Apr'64, Apr'65	4.6	300 (6)	140 (4)	16
3,4	3—6	Hay	12	Jan-Apr'64	4.1	320 (6)	130 (7)	16
5	3	Hay	6	Apr'65	3.7	315 (26)	115 (8)	15
6	4	Hay, roots	9	Jan-Mar'64	3.5	375 (9)	130 (3)	16
7,8	5	Hay, roots	8	May'65	5.4	300 (21)	155 (13)	19
				Means for 93 samples	4.3	317 (4)	137 (2)	17
9	2	Hay, roots "low fat"	10	Apr-May'64	5.2	385 (11)	200 (4)	17
Protein-free feed								
		Test cow No. 1	23	Apr.'63-July'64	5.7	400 (7)	225 (7)	26
		3	24	May'63-June'65	6.4	370 (7)	240 (11)	20
		4	7	Sep-Dec'63	5.1	480 (6)	245 (5)	29
		5	23	Jan'64-Feb,66	5.2	435 (12)	225 (11)	33
		6	8	Apr'65-Feb'66	5.0	435 (10)	230 (8)	26
				Means for 85 samples	5.7	413 (6)	231 (5)	26

Standard errors are given in parentheses.

when the amount of oil in the feed was increased according to the figures shown. Maize-, linseed- and olive-oil were used in compounding the oil ration.

Test and normal feeds : seasonal and lactation period variation in milk cholesterol levels Herd milk cholesterol values (normal feeding) showed a rise at the commencement of pasturage, followed by a steady fall during the succeeding 9 months, values being low at the end of the stall-feeding period (Figure II).

Table III. Effect of oil ration on test milk cholesterol

Daily oil ration per cow, ml.	Number of milk samples	Total cholesterol mg/100 g fat	mg/kg milk
40	27	420	240
50	8	410	215
80	30	410	240
100	7	430	210
120	7	430	235
140	7	380	210

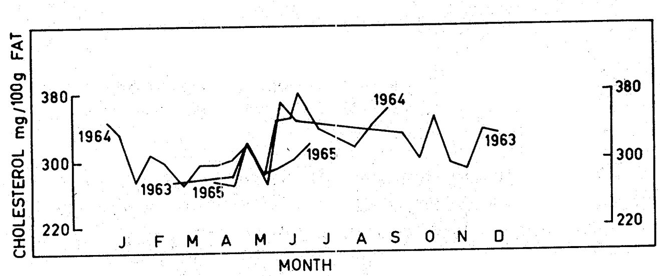

Figure II. Milk fat cholesterol (mg/100 g fat): month-by-month variation with normal feeding. Herd milk, farm 1.

The rise does not seem to be due to the fact that most of the herd were in early lactation in May-June, for early lactation values are low (see Figure IV).

The graphs for 3 of the test cows (Figure III) show that the cholesterol values can vary considerably over a short period. What slight trend there is is towards lower values as the lactation progresses.

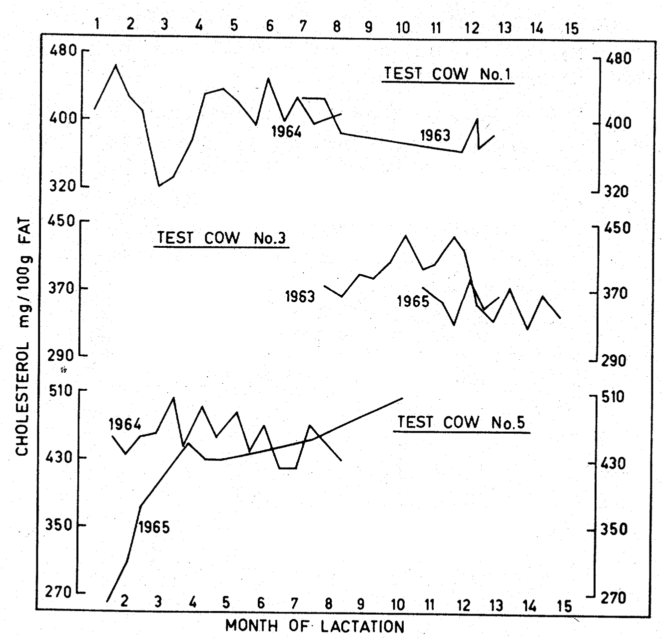

Figure III. Test milk fat cholesterol (mg/100 g fat): variation with stage of lactation.

This is in contrast to the results obtained with single cows on normal feed (Figure IV), where cholesterol values are seen to increase throughout the lactation. Assuming linear relationships between the concentration of cholesterol in the milk fat (y, mg/100 g) or in the whole milk (y', mg/kg) and the duration of the lactation in weeks (x) at the time of sampling, the regression lines are:

$$y = 273 + 1.71\,x \quad (r = 0.458,\ P < 0.1\,\%), \text{ and}$$
$$y' = 128 + 0.54\,x \quad (r = 0.304,\ 0.1\,\% < P < 1.0\,\%).$$

Daily output of cholesterol in milk This averaged 1.3 and 1.8 g for the test and normally-fed cows respectively.

Distribution of cholesterol in milk The test milk showed normal values when the butterfat cholesterol

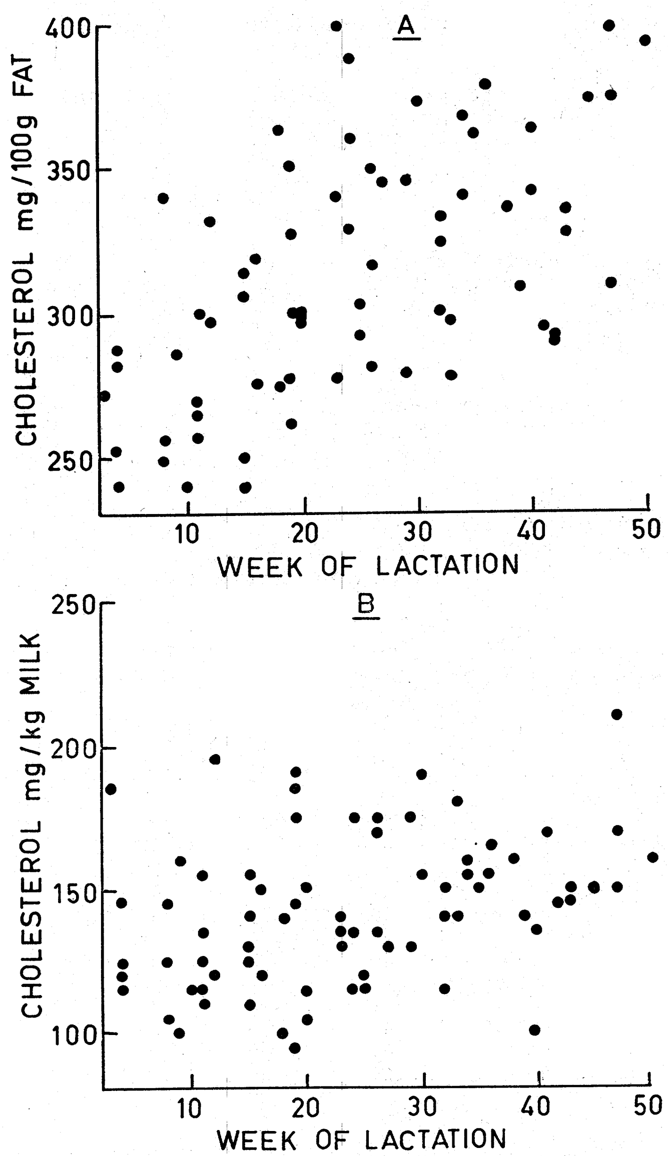

Figure IV. Variation of the level of cholesterol in milk fat (A) and in whole milk (B) with stage of lactation. Normal feeding.

was compared to the Röse-Gottlieb fat cholesterol figure (Table IV).

Table IV. Cholosterol in butterfat and Röse-Gottlieb fat
The cholesterol values are in mg/100 g fat.

Sample		Cholesterol in Butterfat (A)	Cholesterol in Röse-Gottlieb fat (B)	$100 \times \frac{A}{B}$
Herd milk (normal feed)	1	290	355	82
Herd milk (normal feed)	2	260	320	82
Test milk	1	300	350	85
Test milk	2	355	430	83
Test milk	3	330	385	85

Cholesterol in colostrum In the 7 series of colostrum samples analysed, cholesterol values for both test and normally-fed cows were elevated, with highest values in the first milking and decreasing values in subsequent milkings. By the fifth milking the fat cholesterol figure was usually about normal, though the milk cholesterol figure was still high (Table V).

Table V. Cholosterol in colostrum
Values given for each sample are mg/100 g fat and mg/kg milk respectively.

	Milking No. 1	2	3	4	5	6	10
Test cow 1	870	—	790	—	—	—	410
	550		370				360
Test cow 3	—	—	—	—	365	—	—
					255		
Test cow 6	570	475	420	400	355	340	—
	190	265	150	300	305	290	
Test cow 3	640	—	500	—	425	—	—
	450		270		260		
Normally-fed cow 1	770	—	490	—	360	—	—
	210		250		370		
Normally-fed cow 2	810	—	790	570	—	—	—
	690		250	285			
Normally-fed cow 3	1950	—	710	—	510	—	—
	145		290		175		

This research has been financed in part by a grant made by the **United States Department of Agriculture, Agricultural Research Service.**

References (CA = Chemical Abstracts, DSA = Dairy Science Abstracts).

1. Dam, H., Z. physiol. Chem. **224,** 127 (1934).
2. Kritchevsky, D., and Tepper, S. A., J. Nutrition **74,** 441 (1961).
3. Morice, I. M., J. Chem. Soc. 1200 (1951).
4. Klostermann, M., and Opitz, H., Z. Untersuch. Nahrungs- u. Genußmittel **27,** 713 (1914). Cited in reference 6.
5. Fox, F. W., and Gardner, J. A., Biochem. J. **17,** 94 (1923).
6. Ansbacher, S., and Supplee, G. C., J. Biol. Chem. **105,** 391 (1934).
7. Kayser, F., and Tachkova, D., Bull. Soc. Pharm. Nancy **34,** 46 (1957). CA **52,** 20718 (1958).
8. Lobzov, K. I., Trud. Mosk. vet. Akad. **30,** 126 (1960). DSA **25,** 3306 (1963).
9. Mulder, H., and Zuidhof, T. A., Neth. Milk. Dairy J. **12,** 173 (1958).
10. Thompson, M. P., Brunner, J. R., Stine, C. M., and Lindqvist, K., J. Dairy Sci. **44,** 1589 (1961).
11. Nataf, B., Mickelsen, O., Keys, A., and Petersen, W. E., J. Nutrition **36,** 495 (1948).
12. Gulvady, S. L., Kannan, A., and Basu, K. P., Indian J. Dairy Sci. **5,** 125 (1952). DSA **15,** 151 (1953). CA **48,** 2940 (1954).
13. Laskowski, K., Pr. Inst. Przem. mlecz. **8,** 21 (1961). DSA **24,** 2088 (1962).
14. Laskowski, K., Pr. Inst. Przem. mlecz. **9,** 1 (1963). DSA **27,** 604 (1965).
15. Steger, H., Arch. Tierz. **4,** 199 (1961). DSA **24,** 1451 (1962).
16. Rotenberg, S., and Baranowski, S., Acta Physiol. Polon. **16,** 465 (1965). DSA **28,** 1663 (1966).
17. Hartmann, P. E., and Lascelles, A. K., Australian J. Biol. Sci. **17,** 935 (1964).
18. Patton, S., and McCarthy, R. D., J. Dairy Sci. **46,** 396 (1963).
19. Patton, S., and McCarthy, R. D., J. Dairy Sci. **46,** 916 (1963).
20. McCarthy, R. D., and Patton, S., Biochim. Biophys. Acta **70,** 102 (1963).
21. Hirsch, J., and Ahrens, E. H., J. Biol. Chem. **233,** 311 (1958).
22. Patton, S., and McCarthy, R. D., Nature **209,** 616 (1966).
23. McCarthy, R. D., and Patton, S., Nature **202,** 347 (1964).
24. Patton, S., McCarthy, R. D., and Dimick, P. S., J. Dairy Sci. **48,** 1389 (1965).
25. Webster, D., Clin. Chim. Acta **8,** 731 (1963).
26. Zak, B., Am J. Clin. Pathol. **27,** 583 (1957).
27. Riis, P. M., and Moustgaard, J., "Use of Radioisotopes in Anim. Biol. and the med. Sciences" Academic Press, London, New York 1962 **2,** 167. DSA **25,** 2295 (1963).

28. Riis, P. M., Aarsberetn. Inst. Sterilitetsforskn. K. Vet.-Landb Højsk., Kbh. 159 (1961). DSA **24,** 2279 (1962).
29. cf. Virtanen, A. I., Science **153,** 1603 (1966).
30. Kirsten, A., Z. Untersuch. Nahrungs- u. Genußmittel **5,** 833 (1902). Cited in reference 6.
31. Denis, W., and Minot, A. S., J. Biol. Chem. **36,** 59 (1918).
32. Wacker, L., and Beck, K. F., Z. Kinderheilk. **27,** 288 (1921).
33. Nakanishi, H., Nagoja J. Med. Sci. **5,** 190 (1931). CA **26,** 5999 (1932).
34. Achard, Ch., Levy, J., and Georgiakakis, N., Arch. mal. app. digest. **24,** 785 (1934). CA **31,** 3582 (1937).
35. Dam, H., Biochem. Z. **270,** 112 (1934).
36. Torrisi, D., Arch. fisiol. **39,** 431 (1940). CA **34,** 4129 (1940).
37. Kon, S. K., Mawson, E. H., and Thompson, S. Y., Nature **154,** 82 (1944).
38. Marques, A., Rev. Med. vet. Lisboa **40,** 314 (1945). DSA **11,** 100 (1949).
39. Nieman, C., Groot, E. H., and Rooselaar, W. J., Acta Physiol. Pharmacol. Neerl. **1,** 488 (1950). CA **45,** 2598 (1951).
40. Pihl, A., Scand. J. Clin. Lab. Invest. **4,** 115 (1952). CA **46,** 8779 (1952).
41. Ševčíková, M., Výživa Lidu **11,** 44 (1956). DSA **18,** 514 (1956).
42. Lobanov, D. I., Vasil'eva, É. N., and Gorelova, L. D., Vopr. Pitaniya **17,** 39 (1958). DSA **20,** 1446 (1958).
43. Man, J. M. de, Z. Ernährungswiss. **5,** 1 (1964).
44. McCarthy, M. J., Kuksis, A., and Beveridge, J. M. R., J. Lipid Res. **5,** 609 (1964).
45. Williams, J. H., Kuchmak, M., and Witter, R. F., J. Lipid Res. **6,** 461 (1965).
46. Brown, H. H., Zlatkis, A., Zak, B., and Boyle, A. J., Anal. Chem. **26,** 397 (1954).
47. Sperry, W. M., and Webb, M., J. Biol. Chem. **187,** 97 (1950).
48. Schwartz, D. P., Burgwald, L. H., and Brewington, C. R., J. Am. Oil. Chemists' Soc. **43,** 472 (1966).
49. Schwartz, D. P., Brewington, C. R., and Burgwald, L. H., J. Lipid Res. **8,** 54 (1967).
50. Peeters, G., and Lauryssens, M., in "Metabolism and Physiological Significance of Lipids" (Editors Dawson, R. M. C. and Rhodes, D. N.) Wiley, London. 1964. p. 351.
51. Kritchevsky, D., "Cholesterol" Wiley, New York. 1958.
52. Copius Peereboom, J. W., Verslag. Landbouwk. Onderzoek. No. 69.8 (1963).
53. Eisner, J., Wong, N. P., Firestone, D., and Bond, J., J. Assoc. Offic. Agr. Chemists **45,** 337 (1962).
54. Den Herder, P. C., Neth. Milk. Dairy J. **9,** 261 (1955).

Zusammenfassung

HOMER, D. R., und VIRTANEN, A. I.: **Cholesteringehalt der Kuhmilch. Untersuchungen über Milch von Kühen bei normaler und proteinfreier Fütterung.** *„Milchwissenschaft"* **22.** *1—7 (1967).*

21 Cholesterin (Milch).

Es werden Methoden zur Bestimmung des Gehalts des Milchfetts an Gesamt- und Ester-Cholesterin beschrieben. Gesamt-Cholesterin wurde durch Fällung mit Digitonin nach Verseifung und Ester-Cholesterin durch Trennung mit Hilfe einer Kieselgelsäule bestimmt. In beiden Fällen erfolgt die kolorimetrische Bestimmung mit Eisenchlorid und Schwefelsäure.

Die Werte des Cholesteringehalts der Milch, die auf verschiedene Fütterung zurückzuführen sind, wurden während eines Zeitraumes von 3 Jahren berechnet. Die Proben stammten von Ayrshire-Kühen. Die Durchschnittswerte des Cholesteringehalts der Milch normal gefütterter Kühe (93 Proben) betrugen 317 mg/100 g Milchfett und 137 mg/kg Milch und standen in Übereinstimmung mit den Angaben anderer Verfasser. Die entsprechenden Werte bei 5 Kühen auf proteinfreier Fütterung (85 Proben) waren 413 mg/100 g Milchfett und 231 mg/kg Milch.

Diese Unterschiede können mit den heutigen Theorien über den Ursprung der Milchlipoide in der Milchdrüse der Kuh in Übereinstimmung gebracht werden. Nach diesen Theorien ist die Konzentration des Cholesterins in Milchfett ein Indikator des Anteils des Milchdrüsenlipoids, der durch das Cholesterinester-Transesterifizierungssystem gebildet wird.

Im Verlaufe der Laktation wurde eine Zunahme der Konzentration des Cholesterins der Milch und des Milchfetts bei normal gefütterten Kühen beobachtet. Dok.-Ref.

HOMER, D. R., and VIRTANEN, A. I.: **Cow's milk cholesterol. Studies on the milk of cows on normal and protein-free feeds.** *„Milchwissenschaft"* **22.** *1—7 (1967).*

21 Cholesterol (milk).

Methods are detailed for the determination of milk fat total and esterified cholesterol, the former by digitonin precipitation after saponification and the latter by column chromatography on silica gel, using ferric chloride-sulphuric acid colorimetry to estimate both ester and digitonide.

A significant difference in milk cholesterol values associated with different feeds is reported for the first time. Samples were collected from Ayrshire cows over a period of 3 years. The mean milk cholesterol values for cows on normal winter rations or pasture (93 samples) were 317 mg per 100 g milk fat and 137 mg per kg milk, figures similar to those reported by other authors. The corresponding values for 5 cows receiving a protein-free feed (85 samples) were 413 mg per 100 g milk fat and 231 mg per kg milk.

These differences may be brought into accordance with current theories about the origins of milk lipid in the cow mammary gland, according to which the concentration of cholesterol in milk fat may be an index of that proportion of gland lipid metabolised to milk lipid which is processed by the cholesterol ester transesterification system.

For normally fed cows, a rise in the concentration of cholesterol in the milk and milk fat as the lactation progressed was observed.

HOMER, D. R., et VIRTANEN, A. I.: **La cholestérine du lait de vache. Quelques études sur le lait des vaches alimentées au fourrage normal et au fourrage sans protéines.** *„Milchwissenschaft"* **22.** *1—7 (1967).*

21 Cholestérine (lait).

HOMER, D. R., et VIRTANEN, A. I.: **Colesterina de la leche de vaca. Estudios sobre la leche de vacas con alimento normal y alimento sin proteínas.** *„Milchwissenschaft"* **22.** *1—7 (1967).*

21 Colesterina (leche).